AMENDMENT

Kindly amend the application, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, as follows.

IN THE CLAIMS:

Kindly amend the claims, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, to read as follows:

1. (Original) A method for releasing a soluble or membrane associated intracellular protein of interest (POI) from a cell comprising the steps of:

(a) providing a cell comprising a soluble or membrane associated intracellular POI;

(b) contacting the cell with a membrane extracting composition comprising a quarternary ammonium compound; and

(c) causing the POI to be released from the cell under conditions sufficient for the specific release of the POI and in a soluble form.

2. (Original) The method according to claim 1, wherein the quarternary ammonium compound is selected from the group consisting of Lauroyl Trimethyl Ammonium Bromide (LTAB), Myristyl Trimethyl Ammonium Chloride (MTAC), CetylTrimethyl Ammonium Chloride (CTAC), Cetrimide, Cetyl Trimethyl Ammonium Bromide (CTAB), Stearoyl Trimethyl Ammonium Chloride (STAC), Stearoyl Trimethyl Ammonium Bromide (STAB), Benzalkonium Chloride (alkyldimethylbenzylammonium chloride), N-Cetylpyridinium Bromide (N-Hexadecylpyridinium bromide), N-Cetylpyridinium Chloride (N-Hexadecylpyridinium chloride), Benzyl Dimcthyl Tetradecyl Ammonium Chloride, Benzyl Dimcthyl Hexadecyl Ammonium Chloride and a combination of any two or more thereof.

3. (Original) The method according to claim 1, wherein the membrane extracting composition comprises from about 0.05% to about 0.6% by weight of the quarternary ammonium compound.

4. (Original) The method according to claim 3, wherein the membrane extracting composition comprises from about 0.1% to about 0.5% by weight of the quarternary ammonium compound.

5. (Original) The method according to claim 4, wherein the membrane extracting composition comprises from about 0.2% to about 0.45% by weight of the quarternary ammonium compound.

6. (Original) The method according to claim 5, wherein the membrane extracting composition comprises about 0.4% by weight of the quarternary ammonium compound.

7. (Original) The method according to claim 1, wherein the cell is contacted with the membrane extracting composition at temperatures from about 4°C to 40°C.

8. (Original) The method according to claim 7, wherein the cell is contacted with the membrane extracting composition at temperatures from about 20°C to about 30°C.

9. (Original) The method according to claim 8, wherein the cell is contacted with the membrane extracting composition at temperatures from about 25°C.

10. (Currently Amended) The method according to claim 1, wherein the cell is contacted with the membrane extracting composition at a pH ~~optima~~ of from about 2.0 to about 11.0.

11. (Currently Amended) The method according to claim 10, wherein the cell is contacted with the membrane extracting composition at a pH ~~optima~~ of from about 5.0 to about 7.0.

12. (Currently Amended) The method according to claim 11, wherein the cell is contacted with the membrane extracting composition at a pH ~~optima~~ of from about 6.3.

13. (Original) The method according to claim 1, wherein the cell is selected from the group consisting of yeast cells, fungal cells and bacterial cells, preferably from yeast and fungal cells.

14. (Original) The method according to claim 13, wherein the cell is selected from the group consisting of yeast cells and fungal cells.

15. (Original) The method according to claim 1, wherein the cell is a transformed cell.

16. (Original) The method according to claim 1, wherein the cell is transformed with a nucleic acid encoding the POI.

17. (Original) The method according to claim 1, wherein the intracellular POI is produced by recombinant DNA techniques.

18. (Original) The method according to claim 1, wherein the POI is an IL-1ra enzyme.

19. (Original) The method according to claim l, wherein the POI is a glucan lyase enzyme.

20. (Original) The method according to claim 19, wherein the yield of glucan lyase is 1 g/litre or more.

21. (Original) The method according to claim 19, wherein the yield of glucan lyase is 3.5 g/litre or more.

22. (Original) The method according to claim 1, wherein the POI is a HOX enzyme.

23. (Original) The method according to claim 22, wherein the HOX enzyme comprises the amino acid sequence set out in SEQ ID No 22 or a variant, homologue, derivative or fragment thereof.

24. (Original) The method according to claim 22, wherein the HOX enzyme is encoded by a nucleotide sequence set out in SEQ ID No 22 or a variant, homologue, derivative or fragment thereof.

25. (Original) The method according to claim 22, wherein the HOX enzyme is encoded by a nucleotide sequence capable of hybridising to the nucleotide sequence set out in SEQ ID No 22 or a variant, homologue, derivative or fragment thereof or a sequence complementary to the hybridisable sequence.

26. (Currently Amended) The method for screening for mutated cells or transformed cells producing elevated levels of a soluble or membrane associated intracellular POI comprising the steps of:

(a) growing the mutated cells at 30°C;
(b) incubating the mutated cells or transformed cells with the membrane extracting composition comprising a quarternary ammonium compound,
(c) recovering the cell free medium; and
(d) screening the cell free medium for elevated levels of the intracellular POI;

such that the presence of the intracellular ~~POT~~ POI in the cell free medium is indicative that the intracellular POI has been released.

27. (Currently Amended) A membrane extracting composition suitable for specifically releasing a soluble or membrane associated intracellular POI, wherein the composition is contacted with the cell under the following conditions:

(a) a percentage by weight of quaternary ammonium compound from about 0.05% to about 0.6%;

(b) a pH ~~optima~~ of from about 2.0 to about 11.0; and

(c) a temperature ~~optima~~ of from about 4°C to about 40°C;

such that the intracellular POI substantially free of contaminating proteins is obtained.

28. (Original) A method of using a membrane extracting composition comprising a quaternary ammonium compound to selectively release a soluble or membrane associated intracellular POI.

29. (Original) A HOX enzyme producible by a method according to claim 1, wherein the HOX enzyme is encoded by a nucleotide sequence set out in SEQ ID No 22 or a variant, homologue, derivative or fragment thereof, or a sequence complementary to the hybridisable sequence, and wherein the nucleotide sequence is synthesised by the oligonucleotides as set out in SEQ ID Nos 2-22.

30. (Original) A POI as defined in claim 1, wherein the POI is released in a substantially non-glycoslyated form from a eukaryotic host organism.

31. (Original) A substantially non-glycosylated POI released from a eukaryotic host organism.

32. (Original) A substantially non-glycosylated POI released from a eukaryotic host organism, wherein the POI is released by the method of claim 1.